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Renal, cardiovascular and endocrine responses of fetal sheep at 0.8 of gestation to an infusion of amino acids

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Amino acid infusions increase renal blood flow (RBF) and glomerular filtration rate (GFR) and stimulate tubular reabsorption in adults. To characterize the effects of amino acids on fetal renal haemodynamics, tubular sodium reabsorption, acid–base homeostasis and plasma renin levels, 11 chronically catheterized fetal sheep aged 121 ± 1 days (term ~150 days) were infused I.V. for 4 h with alanine, glycine, proline and serine (0.1, 0.1, 0.06 and 0.06 mmol min^{-1}, respectively) in 0.15 M saline at 0.165 ml min^{-1}. Eight control fetuses were given saline. During amino acid infusion, plasma amino acid levels increased up to 20-fold ($P < 0.005$). GFR increased by 50 ± 8 % ($P < 0.001$); there was only a small transient increase in RBF. Proximal fractional sodium reabsorption fell from 74.6 ± 2.9 to 55.5 ± 5.4 % ($P < 0.005$). Distal sodium delivery increased, but a smaller percentage of this distal sodium load was reabsorbed ($P < 0.005$). Thus fractional sodium reabsorption fell from 95.5 ± 0.9 to 81.4 ± 2.0 % ($P < 0.005$). There was a large diuresis, natriuresis, kaliuresis and increase in osmolar excretion ($P < 0.005$). Plasma sodium and chloride concentrations fell ($P < 0.005$). Plasma osmolality did not change. Plasma renin levels fell ($P < 0.05$), cortisol levels increased ($P < 0.05$), and there was a compensated metabolic acidosis. Thus the fetal sheep kidney demonstrated a remarkable functional capacity to respond to amino acid infusion. The increase in filtration fraction and the lack of an increase in RBF suggest that efferent arteriolar vasoconstriction occurred, a very different response from the renal vasodilatation seen in adult animals.

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In adult animals and humans, glomerular filtration rate (GFR) and renal blood flow (RBF) rise by as much as 50 % following ingestion of a protein-rich meal or acute intravenous infusion of amino acids (Shannon *et al.* 1932; ter Wee *et al.* 1985; Hostetter, 1986; Woods *et al.* 1986). The elevation in GFR is dose dependent (Rodriguez-Iturbe *et al.* 1988), and is generally associated with a reduction in renal vascular resistance (ter Wee *et al.* 1985; Hostetter, 1986).

During pregnancy, changes in maternal protein intake could influence not only maternal renal function but also that of the fetal kidney. The renal functional response to amino acids is present in fetal sheep as young as 126 days gestation (term being ~150 days). Woods *et al.* (1996) showed that infusions of amino acids were associated with dose-dependent increases in GFR in parallel with increases in plasma α-amino nitrogen levels. Amino acid reabsorption and sodium reabsorption were also stimulated.

It is not known, however, whether the renal response to amino acids in the fetus in late gestation also involves the characteristic increase in RBF that occurs in the adult in response to protein or amino acids. Measurement of both GFR and RBF would allow the calculation of filtration fraction, and give an indication of whether the mechanism underlying the changes to glomerular haemodynamics was vasodilatory in nature, as it is in the adult. Knowledge of whether the increases in sodium reabsorption were predominantly proximal, or occurred in both the proximal and distal regions of the nephron, would also provide a clearer understanding of the nature of the fetal renal response to amino acids.

In addition, it is not known whether amino acids affect the activity of the circulating fetal renin-angiotensin system, indeed the effects of amino acids on the adult renin-angiotensin system are unclear. Elevations in plasma renin activity (PRA) were observed during chronic high levels of dietary protein in rats and humans (Paller & Hostetter, 1986; Daniels & Hostetter, 1990) and, in adult dogs, PRA increased slightly during amino acid infusion (Woods *et al.* 1986). Other reports indicate that the renin-angiotensin system is not affected by amino acid infusion or a meat meal (Ruilope *et al.* 1987; Wada *et al.* 1991; Woods, 1993*b*). However, since angiotensin II is intimately involved in regulating fetal glomerular haemodynamics, both directly by maintaining

glomerular filtration through efferent arteriolar vasoconstriction (Lumbers *et al.* 1993), and indirectly through its effects on arterial pressure, it was important to see whether increased activity of the renin-angiotensin system might accompany any increase in GFR or RBF.

In the present study we aimed to further characterize the renal response to amino acids in the 121-day-old fetal sheep in order to answer these three questions.

METHODS

The effects of a 4 h fetal intravenous infusion of either an amino acid mixture or saline were examined in 19 chronically catheterized pregnant ewes and their fetuses. These experiments were approved by the Animal Care and Ethics Committee of the University of New South Wales. Ewes were killed at the end of the study by I.V. injection of 3–3.5 g pentobarbitone sodium (Ilium Pentabarb; Troy Laboratories, Smithfield, NSW, Australia).

Surgical preparation

Ewes were fasted for 16 h and anaesthetized with 1 g sodium thiopentone I.V. (Pentothal; Abbott, Kurnell, NSW, Australia). Anaesthesia was maintained with 2–3 % halothane (Fluothane; ICI, Macclesfield, Cheshire, UK) in oxygen. Under aseptic conditions, polyvinyl catheters (i.d. 1.0 mm, o.d. 1.5 mm, 150 cm length), filled with heparinized 0.15 M saline (100 i.u. heparin ml^{-1}, Heparin Injection BP; David Bull Laboratories, Mulgrave, VIC, Australia) were inserted into a fetal femoral artery and both tarsal veins and into the bladder via a suprapubic route. A Doppler flow probe (1.8 mm i.d. cuff, subminature 20 MHz piezoelectric transducer; Iowa Doppler Products, USA) was placed around the left renal artery. A polyvinyl catheter (i.d. 1.5 mm, o.d. 2.7 mm) was placed in the amniotic cavity to measure intra-amniotic pressure and 600 mg of procaine penicillin and 750 mg of dihydrostreptomycin sulfate (Hydropen, Bomac Laboratories, Asquith, NSW, Australia) were injected into the amniotic cavity (Lumbers & Stevens, 1983). Polyvinyl catheters (i.d. 1.5 mm, o.d. 2.7 mm) were inserted into a maternal femoral artery and vein. At the end of surgery, 600 mg of procaine penicillin and 750 mg of dihydrostreptomycin sulfate were given I.M. to the ewe. For the next 2 days these antibiotics were given to the fetus via the amniotic catheter. All arterial and venous catheters were flushed with heparinized saline daily. Ewes were housed in metabolic cages in a room maintained between 18 and 23 °C. They were given free access to water and to 1200 g lucerne chaff, 300 g oats and 6 g sodium chloride daily. Daily fluid and food intake and urine output were measured. No experiments were performed until at least 4 days after surgery.

Experimental protocol

On the day of an experiment, the fetal bladder catheter was opened and urine allowed to drain under gravity for 45 min prior to commencing the experiment. Fetal urine was collected anaerobically under liquid paraffin. Half an hour before the experiment began, the ewe was given 150 μmol kg^{-1} of lithium chloride I.V. The fetus was given I.V. loading doses of lithium chloride (250 μmol kg^{-1}) and ^{125}I-labelled sodium iothalamate (1.8 μCi kg^{-1}; Amersham, UK), followed by a continuous I.V. infusion for the duration of the experiment through a sterile filter (0.2 μm pore size; Minisart, Sartorius, Germany) of 10 μmol kg^{-1} h^{-1} and 0.3 μCi kg^{-1} h^{-1}, respectively, in 0.15 M saline at 0.95 ml h^{-1}.

Maternal and fetal arterial pressures and intra-amniotic pressure were recorded continuously using pressure transducers (Easyvent, Deadender Cap, Ohmeda, BOC) connected to a polygraph (Model 79D, Grass Instrument Co., Quincy, MA, USA). RBF was measured continuously using a 545C-4 directional pulsed Doppler flowmeter (Bioengineering, University of Iowa, IO, USA). RBF, arterial and intra-amniotic pressures were collected every minute using an IBM compatible PC and a Metrabyte DAS16 interface card (Keithley, MA, USA).

Following a 2 h control period, 11 fetal sheep aged 121 ± 1 days gestation were infused I.V. for 4 h with a mixture of alanine, glycine, proline and serine (Sigma). The amino acids were dissolved in 0.15 M saline and infused into a tarsal vein at a combined rate of 0.32 mmol min^{-1} (0.1, 0.1, 0.06 and 0.06 mmol min^{-1}, respectively), at 0.165 ml min^{-1} (Woods *et al.* 1996). This was a pharmacological dose, chosen to be consistent with the study by Woods *et al.* (1996). Eight control fetuses aged 120 ± 1 days gestation were infused for 4 h with sterile 0.15 M saline at the same rate. Animals were allocated to treatment groups prior to surgery. Infusions were prepared on the day of use and were delivered through a 0.2 μm filter (Minisart) using a Braun Perfusor VII pump.

Twelve consecutive 30 min urine collections were made consisting of four control periods followed by eight infusion periods. Fetal arterial blood samples were taken at the midpoint of the second (3 ml), fourth (6 ml), eighth (3 ml) and final collection periods (6 ml). Maternal arterial blood (5 ml) was sampled at the midpoint of the fourth and final periods.

Biochemical analysis

Arterial P_{O_2}, P_{CO_2} and pH were measured at 37 °C and corrected to 39.5 °C using a Ciba-Corning Blood Gas System (Model 288; Medford, MA, USA). Haematocrit was measured in duplicate using a microhaematocrit centrifuge and reader (Hettich, Tuttlingen, Germany). The remaining blood was centrifuged for 10 min at 1083 *g*, 4 °C in tubes containing heparin (20 i.u. per ml blood), and plasma and urine samples were stored at −20 °C until biochemical analysis was carried out.

Urinary and plasma sodium and potassium levels were measured using a Radiometer flame photometer (Model FLM 3; Radiometer Copenhagen, Denmark). Plasma concentrations of chloride, glucose and lactate were measured using an ABL700 Series Analyser (Radiometer). Osmolality of plasma and urine was measured by freezing point depression using a Fiske One-Ten osmometer (Needham Heights, MA, USA). GFR was measured as the renal clearance of ^{125}I-labelled sodium iothalamate in the periods during which blood samples were taken. The concentration of ^{125}I-labelled sodium iothalamate in plasma and urine was determined from the activity of ^{125}I measured using a Packard Auto Gamma counter (model 5650; Downers Grove, IL, USA). Lithium concentrations in plasma and urine were measured using a Perkin-Elmer 272 atomic absorption spectrophotometer (Norwalk, CT, USA). Urinary pH and bicarbonate, titratable acid, ammonium and net acid excretion were determined using methods described by Györy & Edwards (1967) and Györy *et al.* (1974).

Plasma renin levels were determined as the rate of formation of angiotensin I (Ang I) when 100 μl of fetal plasma was incubated with 100 μl of nephrectomised sheep plasma at pH 7.5 and 37 °C for 2 h. Ang I was measured by radioimmunoassay (Lumbers & Lee Lewes, 1979). Plasma cortisol was measured in duplicate

180 μl samples by a coated tube radioimmunoassay using a commercial kit (Spectra, Orion Diagnostica, Finland), following separation from its binding globulins using a dichloromethane extraction procedure (Bocking *et al.* 1986).

Alanine, glycine, proline, serine and urea were measured in 200 μl of plasma by ion-exchange chromatography. Prior to measurement, 20 μl 30 % (w/v) sulfosalicylic acid (Sigma), and 10 μl 5.056 mM β-alanine as an internal standard, were added to the samples, which were then vortexed and centrifuged at room temperature for 5 min at 13 000 r.p.m. One hundred microlitres of supernatant were withdrawn and mixed with 100 μl Beckman Li-A buffer. The sample was then diluted 1:1 with Beckman Li-S buffer and analysed for concentrations of individual amino acids and urea using a Beckman System 6300 high performance amino acid analyser. All amino acid analysis buffers were purchased from Beckman Instruments Inc. (Palo Alto, CA, USA).

Analysis of data

Fetal arterial pressure was corrected for intra-amniotic pressure to obtain true pressure. At the time of each experiment, fetal body weight was estimated from gestational age using a formula derived from the body weights and ages of 46 Australian merino fetuses (Smith, 1982). Fractional reabsorption of sodium by the proximal and distal tubules was calculated from the renal clearance of lithium, where the fractional reabsorption of sodium by the proximal tubule ($FR_{Na,P}$; %) was calculated using the formula:

$$FR_{Na,P} = (1 - \text{clearance of lithium/GFR}) \times 100 \quad (1)$$

(Lumbers *et al.* 1988).

Plasma bicarbonate concentrations were calculated from the measured arterial P_{CO_2} and pH, using a formula derived from the Henderson-Hasselbach equation (Armentrout *et al.* 1977). Filtration fraction relative to control was calculated using the formula:

$$\text{Filtration fraction} = GFR_c/RBF_c, \quad (2)$$

where GFR_c and RBF_c are GFR and RBF expressed as a percentage of their respective control values.

For most variables, data were averaged to obtain a single value for each variable for the 2 h control period. Data are reported for the control period, and for the fourth and final infusion periods ('2 h' and '4 h', respectively). Results are reported as means ± standard error of the mean (S.E.M.), with $n = 11$ for amino acid-infused fetuses and $n = 8$ for control fetuses unless otherwise stated. Within each treatment group, the means for each period were compared using SPSS (SPSS/PC; SPSS Inc., Chicago, IL, USA) either by two-way analysis of variance (ANOVA; Zar, 1984), or by Student's paired *t* test. A Student-Newman-Keuls test was used to determine which period means were different when an ANOVA reached significance (Zar, 1984). Cortisol levels were compared within each treatment group by a Wilcoxon paired sample test (Zar, 1984). Regression equations were determined by the method of least squares using SPSS.

RESULTS

Fetal plasma amino acid levels

Plasma levels of the infused amino acids (alanine, glycine, proline and serine; AGPS) were measured in five amino acid-infused fetuses during control and after 4 h of infusion. The infusion of AGPS resulted in substantial increases in the plasma levels of these amino acids (Fig. 1).

Fetal arterial pressure and heart rate

Systolic pressure increased from control values of 57 ± 1 mmHg during the first 1.5 h of amino acid infusion; it was highest in the first 30 min of the infusion (61 ± 1 mmHg, $P < 0.05$) but had returned to control levels by 2 h. Diastolic pressure did not change from control values of 32 ± 1 mmHg; there was a small increase in mean arterial pressure at 0.5 h from control values of 42 ± 1 mmHg to 44 ± 1 mmHg ($P < 0.05$). There was a progressive bradycardia throughout AGPS infusion, even after arterial pressure had returned to baseline, from 181 ± 4 beats min^{-1} during control to 161 ± 3 beats min^{-1} in the last 30 min studied ($P < 0.005$). By contrast, systolic pressure fell during saline infusion, from control values of 58 ± 1 mmHg to a nadir of 55 ± 2 mmHg after 2–2.5 h ($P < 0.005$), and heart rate increased to 197 ± 4 and 193 ± 5 beats min^{-1} during the first and second half-hour periods, respectively, from control values of 184 ± 3 beats min^{-1} ($P < 0.05$). Neither diastolic nor mean pressure changed from control values of 34 ± 1 and 43 ± 1 mmHg, respectively.

Fetal arterial blood gases, haematocrit and plasma composition

Arterial P_{O_2} and P_{CO_2} did not change during either AGPS or saline infusion. Arterial pH fell slightly by 2 h of AGPS infusion, but returned to control levels by 4 h. The fall in arterial pH at 2 h was associated with an increase in plasma lactate levels and a decrease in plasma bicarbonate concentrations; both of these changes were sustained. During saline infusion, arterial pH and lactate levels remained similar to control values. However, plasma bicarbonate concentrations were decreased at both 2 h and 4 h (Table 1).

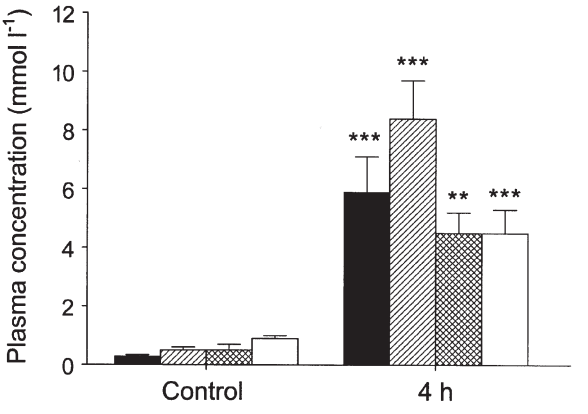

Figure 1. Plasma concentrations of AGPS

Plasma levels of alanine (■), glycine (▨), proline (▩) and serine (□) in 5 fetal sheep during control and after 4 h of infusion of AGPS. Student's paired *t* test: ** $P < 0.01$, *** $P < 0.005$ *vs.* Control.

Table 1. Fetal arterial blood gases, pH, haematocrit and plasma composition

	Saline			Amino acids		
	Control	2 h	4 h	Control	2 h	4 h
P_{O_2} (mmHg)	18.6 ± 1.4	18.5 ± 0.9	18.0 ± 1.2	21.1 ± 0.8	20.9 ± 0.7	20.4 ± 0.7 (10)
P_{CO_2} (mmHg)	54.1 ± 0.9	53.2 ± 1.1	54.2 ± 0.9	53.7 ± 1.1	53.7 ± 1.2	53.0 ± 1.0 (10)
Arterial pH	7.35 ± 0.01	7.33 ± 0.01	7.32 ± 0.01	7.33 ± 0.01	7.30 ± 0.01 *	7.32 ± 0.02 (10)
Haematocrit (%)	32.1 ± 1.1	31.5 ± 1.3	29.9 ± 1.2 ***†††	31.2 ± 1.3	30.1 ± 1.3 ***	29.5 ± 1.2 ***†
Plasma:						
Osmolality (mosmol kg^{-1})	290 ± 3	287 ± 4	285 ± 2	298 ± 4	300 ± 6	294 ± 2
[Sodium] (mmol l^{-1})	143 ± 1	144 ± 1	145 ± 1	148 ± 2	144 ± 2 *	140 ± 2 ***†††
[Chloride] (mmol l^{-1})	110 ± 1 (6)	110 ± 1 (6)	109 ± 4 (6)	109 ± 1 (9)	104 ± 1 *** (8)	101 ± 1 ***††† (9)
[Potassium] (mmol l^{-1})	3.9 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	4.1 ± 0.2	4.1 ± 0.2	4.0 ± 0.1
[Bicarbonate] (mmol l^{-1})	28.1 ± 0.3	26.8 ± 0.3 ***	26.6 ± 0.4 ***	27.3 ± 0.8	25.0 ± 0.6 ***	25.9 ± 0.9 **† (10)
[Glucose] (mmol l^{-1})	0.9 ± 0.1 (6)	0.9 ± 0.1 (6)	0.9 ± 0.1 (6)	1.0 ± 0.1 (9)	1.1 ± 0.1 (8)	1.1 ± 0.1 (9)
[Lactate] (mmol l^{-1})	1.3 ± 0.1 (6)	1.2 ± 0.1 (6)	1.3 ± 0.1 (6)	1.3 ± 0.1 (9)	2.5 ± 0.3 *** (8)	2.6 ± 0.3 *** (9)
[Urea] (mmol l^{-1})	—	—	—	7.8 ± 0.8 (5)	—	7.0 ± 0.5 (5)
$P_{Na/K}$	36.6 ± 1.1	38.3 ± 1.1 *	37.8 ± 1.2	36.6 ± 1.5	35.8 ± 1.1	35.1 ± 1.1

Values are means ± S.E.M. during the control period and after 2 and 4 h of infusion of either saline or amino acids ($n = 8$ and $n = 11$, respectively, unless otherwise stated in parentheses). P_{O_2}, arterial oxygen tension; P_{CO_2}, arterial carbon dioxide tension; $P_{Na/K}$, plasma sodium to potassium ratio. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ *vs.* control period; † $P < 0.05$, ††† $P < 0.005$ *vs.* 2 h (Student-Newman-Keuls test).

Haematocrit fell progressively during AGPS infusion, and in saline-infused fetuses it was lower than control at 4 h. Fetal plasma osmolality did not change during AGPS infusion, but in each fetus there was a progressive fall in plasma sodium and chloride. Plasma potassium, glucose and urea levels did not change, nor did the plasma sodium to potassium ratio. During saline infusion, only the plasma sodium to potassium ratio changed; it increased over the first 2 h (Table 1).

Plasma renin levels

Plasma renin levels were measured in six control fetuses and five amino acid-infused fetuses. During AGPS infusion, renin levels fell in each fetus from a mean of 9.3 ± 2.1 ng ml^{-1} h^{-1} during control to 4.7 ± 1.5 ng ml^{-1} h^{-1} after 4 h of infusion ($P < 0.05$). After 4 h of saline infusion, plasma renin levels were 4.7 ± 1.4 ng ml^{-1} h^{-1}; these values were not significantly different from control values of 5.9 ± 1.6 ng ml^{-1} h^{-1}.

Plasma cortisol levels

Plasma cortisol levels were measured in five fetuses from each treatment group. They increased by 388 ± 117 % of control values during amino acid infusion (from 3.6 ± 0.7 to 18.2 ± 6.1 nmol l^{-1}, $P < 0.05$; Wilcoxon paired sample test). In saline-infused animals, cortisol levels remained at control values of 3.3 ± 1.0 nmol l^{-1}.

Fetal renal function

After 2 h of AGPS infusion, GFR had increased by 50 ± 8 % ($P < 0.001$; Fig. 2*A*), and it remained high. In comparison to this large, sustained rise in GFR, there was a relatively small increase in RBF by 2 h (12 ± 3 %, $P < 0.05$; Fig. 2*B*), which returned to control levels for the remainder of the infusion. The greater magnitude of the GFR response compared with that of RBF was reflected in an increase in filtration fraction throughout the amino acid infusion (Fig. 2*C*). During saline infusion there were no significant changes in GFR, RBF or filtration fraction, although RBF tended to fall (Fig. 2).

In both groups of fetuses, there was a strong positive linear relationship between GFR measured at 2 and 4 h during the infusion period (GFR_{inf}) and GFR measured during control ($GFR_{control}$). In the eight saline-treated fetuses, this relationship was described by the equation:

$$GFR_{inf} = 0.90GFR_{control} + 0.16 \quad (3)$$

($r^2 = 0.90, P < 0.001, n = 16$),

while in the 11 amino acid-infused fetuses, the relationship was:

$$GFR_{inf} = 1.03GFR_{control} + 1.24 \quad (4)$$

($r^2 = 0.78, P < 0.001, n = 22$).

Furthermore, in amino acid-infused fetuses there was an inverse relationship between the increase in GFR expressed as a percentage of control GFR ($\Delta GFR_{\%control}$) and control GFR ($r^2 = 0.47$, $P < 0.001$, $n = 22$). That is, the relative increase in GFR was greater in fetuses that had a low GFR prior to their infusion; this was described by the equation:

$$\Delta GFR_{\%control} = e^{((0.89/GFR_{control}) + 4.65)}. \quad (5)$$

No such relationship existed in saline-infused fetuses.

There was a large progressive rise in urine flow rate during amino acid infusion, which did not occur in saline-infused fetuses (Fig. 3). Accompanying this diuresis were progressive increases in urinary osmolality, renal osmolar excretion and osmolar clearance (Table 2). Renal excretion rates of sodium and potassium were also increased throughout infusion, as was the urinary sodium to potassium ratio (Table 2). Urinary osmolality, renal excretion rates and the urinary sodium to potassium ratio did not change with saline infusion (Table 2).

Rates of sodium reabsorption rose during AGPS infusion, but not by enough to compensate for the increase in filtered sodium load, thus there was a progressive fall in fractional sodium reabsorption (Table 3). While both proximal and distal tubular sodium reabsorption increased, the increase in proximal tubular reabsorption was small (only ~25 % at 2 h) compared with the increase in filtered sodium load (~40 % at 2 h). Thus proximal fractional sodium reabsorption fell, and more sodium was delivered to the distal nephron. By contrast, the fraction of the filtered load of sodium reabsorbed by the distal tubule did not change, although the distal tubule reabsorbed a smaller fraction of the increased load of sodium delivered to it (Table 3).

Potassium reabsorption increased during AGPS infusion, so that the fractional reabsorption of potassium did not

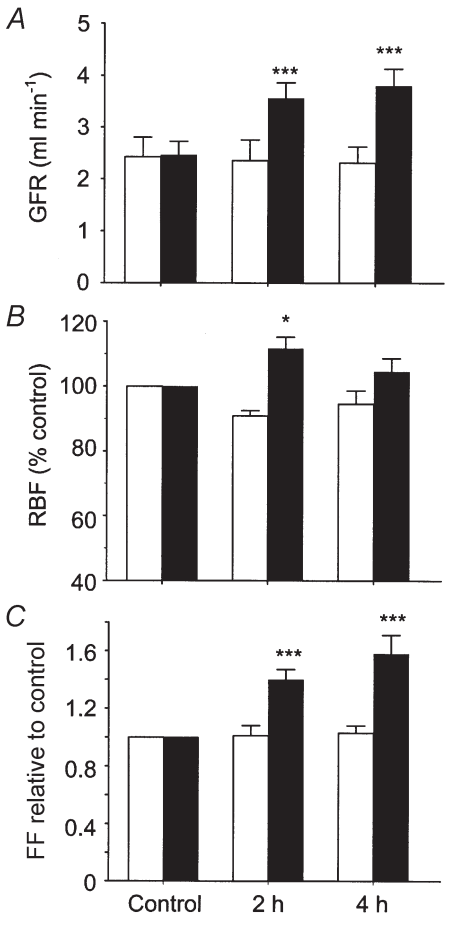

Figure 2. Renal haemodynamics

Fetal glomerular filtration rate (GFR; *A*), renal blood flow relative to control (RBF; *B*) and filtration fraction relative to control (FF; *C*) during the control period, and after 2 and 4 h of an infusion of isotonic saline (□, $n = 8$ for GFR, $n = 5$ for RBF and FF) or amino acids (■, $n = 11$ for GFR, $n = 9$ for RBF and FF). Values are means ± S.E.M. * $P < 0.05$, *** $P < 0.001$ *vs.* control period (Student-Newman-Keuls test).

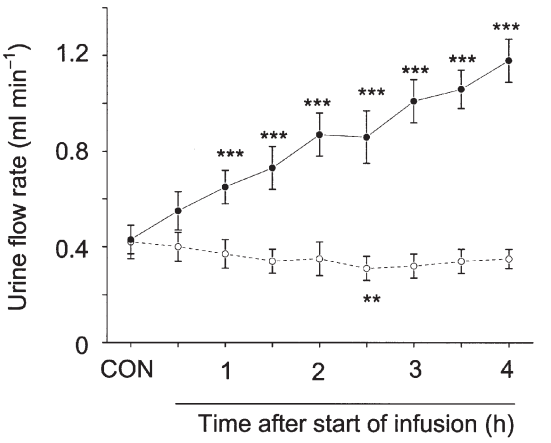

Figure 3. Fetal urine flow rate

Mean ± S.E.M. urine flow rate during control (CON) and during infusion of saline (○, $n = 8$) or amino acids (●, $n = 11$). ** $P < 0.01$, *** $P < 0.001$ *vs.* control period (Student-Newman-Keuls test).

Table 2. Urinary osmolality, free water clearance and renal excretion rates

	Saline			Amino acids		
	Control	2 h	4 h	Control	2 h (10)	4 h
Osmolality (mosmol kg^{-1})	120 ± 4	138 ± 13	125 ± 5	138 ± 13	204 ± 19 ***	235 ± 16 ***†
C_{osmol} (ml min^{-1})	0.17 ± 0.03	0.16 ± 0.03	0.15 ± 0.02	0.19 ± 0.04	0.58 ± 0.07 ***	0.95 ± 0.10 ***†††
C_{H_2O} (ml min^{-1})	0.26 ± 0.05	0.19 ± 0.05	0.20 ± 0.03	0.22 ± 0.04	0.29 ± 0.07	0.23 ± 0.07
E_{osmol} (μosmol min^{-1})	49 ± 8	45 ± 7	43 ± 5	60 ± 12	175 ± 23 ***	279 ± 29 ***†††
E_{Na} (μmol min^{-1})	13 ± 2	12 ± 2	12 ± 1	19 ± 5	62 ± 11 ***	97 ± 11 ***†††
E_K (μmol min^{-1})	2.2 ± 0.5	1.7 ± 0.3	1.7 ± 0.4	4.0 ± 1.1	6.5 ± 1.7 ***	6.8 ± 1.3 ***
$U_{Na/K}$	12.0 ± 5.8	12.0 ± 4.5	12.7 ± 4.6	8.1 ± 2.1	18.9 ± 5.9 ***	19.5 ± 3.7 ***

Values are means ± S.E.M. during the control period and after 2 and 4 h of infusion of either isotonic saline ($n = 8$) or amino acids ($n = 11$; the number of fetuses is stated in parentheses if different from these values). C_{osmol}, osmolar clearance; C_{H_2O}, free water clearance; E_{osmol}, osmolar excretion; E_{Na} and E_K, excretion of sodium and potassium, respectively; $U_{Na/K}$, urinary sodium to potassium ratio. *** $P < 0.005$ *vs.* control period; † $P < 0.05$, ††† $P < 0.005$ *vs.* 2 h (Student-Newman-Keuls test).

Table 3. Tubular handling of sodium and potassium

	Saline			Amino acids		
	Control	2 h	4 h	Control	2 h	4 h
$Filt_{Na}$ (μmol min^{-1})	346 ± 54	338 ± 56	333 ± 43	366 ± 41	518 ± 47 ***	536 ± 50 ***
R_{Na} (μmol min^{-1})	332 ± 52	325 ± 55	321 ± 43	348 ± 38	453 ± 43 *** (10)	438 ± 43 ***
FR_{Na} (%)	95.9 ± 0.8	96.3 ± 0.6	96.3 ± 0.5	95.5 ± 0.9	88.7 ± 1.3 *** (10)	81.4 ± 2.0 ***†††
$R_{Na,P}$ (μmol min^{-1})	256 ± 43	253 ± 45	254 ± 36	270 ± 31	339 ± 45 *	309 ± 45
$FR_{Na,P}$ (%)	73.5 ± 2.0	74.8 ± 2.7	76.5 ± 2.6	74.6 ± 2.9	64.4 ± 4.7 *	55.5 ± 5.4 ***†
DD_{Na} (μmol min^{-1})	90 ± 15	85 ± 17	79 ± 15	96 ± 16	179 ± 26 ***	227 ± 24 ***†
$R_{Na,D}$ (μmol min^{-1})	76 ± 14	73 ± 15	67 ± 15	78 ± 13	111 ± 25 (10)	130 ± 20 *
$FR_{Na,D}$ (%)	22.4 ± 2.2	21.5 ± 2.8	19.8 ± 2.6	20.9 ± 2.7	23.4 ± 5.1 (10)	25.9 ± 4.3
$\%DD_{Na,R}$ (%)	83.3 ± 3.2	83.6 ± 3.7	83.6 ± 2.3	81.2 ± 3.5	61.3 ± 6.4 *** (10)	55.2 ± 5.8 ***
$Filt_K$ (μmol min^{-1})	9.3 ± 1.2	8.7 ± 1.3	8.7 ± 0.9	10.4 ± 1.4	14.8 ± 1.6 ***	15.5 ± 1.7 ***
R_K (μmol min^{-1})	7.0 ± 1.6	7.0 ± 1.6	7.1 ± 1.2	6.5 ± 0.7	8.4 ± 0.8 * (10)	8.7 ± 0.9 *
FR_K (%)	70.4 ± 8.0	73.5 ± 7.4	77.4 ± 6.3 ***†	67.7 ± 6.1	61.6 ± 7.4 (10)	57.9 ± 5.9

Values are means ± S.E.M. during the control period and after 2 and 4 h of infusion of either isotonic saline ($n = 8$) or amino acids ($n = 11$; the number of fetuses is stated in parentheses if different from these values). Filt, filtered load; *R* and FR, absolute and fractional reabsorption, respectively; $R_{Na,P}$ and $FR_{Na,P}$, absolute and fractional reabsorption of sodium by the proximal tubule, respectively; $R_{Na,D}$ and $FR_{Na,D}$, absolute and fractional reabsorption of sodium by the distal tubule, respectively; DD_{Na}, distal delivery of sodium; $\%DD_{Na,R}$, percentage of distal delivery of sodium reabsorbed by the distal tubule. * $P < 0.05$, *** $P < 0.005$ *vs.* control period; † $P < 0.05$, ††† $P < 0.005$ *vs.* 2 h (Student-Newman-Keuls test).

Table 4. Sodium reabsorption and GFR

A.	Saline infusion			
	Control	$R_{Na} = 137.39 \times GFR + 0.01$	$r^2 = 0.995$	$P < 0.001$
	2 h	$R_{Na} = 136.31 \times GFR + 5.22$	$r^2 = 0.995$	$P < 0.001$
	4 h	$R_{Na} = 137.90 \times GFR + 3.03$	$r^2 = 0.991$	$P < 0.001$
	Control	$R_{Na,P} = 111.78 \times GFR - 13.90$	$r^2 = 0.959$	$P < 0.001$
	2 h	$R_{Na,P} = 109.28 \times GFR - 4.08$	$r^2 = 0.942$	$P < 0.001$
	4 h	$R_{Na,P} = 111.55 \times GFR - 3.21$	$r^2 = 0.929$	$P < 0.001$
	Control	$R_{Na,D} = 25.62 \times GFR + 13.91$	$r^2 = 0.458$	$P = 0.06$
	2 h	$R_{Na,D} = 27.03 \times GFR + 9.30$	$r^2 = 0.490$	$P = 0.05$
	4 h	—	$r^2 = 0.303$	n.s.
B.	Amino acid infusion			
	Control	$R_{Na} = 144.80 \times GFR - 8.30$	$r^2 = 0.960$	$P < 0.001$
	2 h	$R_{Na} = 126.77 \times GFR + 1.16$	$r^2 = 0.961$	$P < 0.001$
	4 h	$R_{Na} = 127.91 \times GFR - 47.50$	$r^2 = 0.949$	$P < 0.001$
	Control	$R_{Na,P} = 111.72 \times GFR - 4.59$	$r^2 = 0.860$	$P < 0.001$
	2 h	$R_{Na,P} = 121.67 \times GFR - 94.54$	$r^2 = 0.666$	$P < 0.005$
	4 h	$R_{Na,P} = 119.18 \times GFR - 143.83$	$r^2 = 0.741$	$P < 0.001$
	Control	$R_{Na,D} = 33.09 \times GFR - 3.71$	$r^2 = 0.418$	$P < 0.05$
	2 h	—	$r^2 = 0.005$	n.s.
	4 h	—	$r^2 = 0.021$	n.s.

Relationships between total sodium reabsorption (R_{Na}) and GFR, reabsorption of sodium by the proximal tubule ($R_{Na,P}$) and GFR, and reabsorption of sodium by the distal tubule ($R_{Na,D}$) and GFR in fetuses infused with saline (A), or with amino acids (B), during control, and at 2 and 4 h of infusion.

change (Table 3). In saline-infused fetuses, fractional potassium reabsorption was increased at 4 h. Otherwise, there were no changes in the renal handling of sodium and potassium during saline infusion.

During the control period in saline-infused fetuses, there was a strong positive relationship between total sodium reabsorption and GFR (Fig. 4*A*, Table 4), largely due to the strong direct relationship between proximal sodium reabsorption and GFR (Fig. 4*B*, Table 4). Sodium reabsorption by the distal tubule was also directly related to GFR, but more weakly (Fig. 4*C*, Table 4). The 4 h saline infusion did not change the strengths of these relationships (Fig. 4, Table 4).

During the control period in fetuses that received amino acids, the relationships between GFR and total, proximal and distal sodium reabsorption were similar to those of saline-infused fetuses (Fig. 5, Table 4). However, during amino acid infusion these relationships changed. The relationship between total sodium reabsorption and GFR, and that between proximal reabsorption and GFR, shifted progressively towards the right. That is, at any level of GFR, less sodium was reabsorbed by both the proximal tubule and the entire nephron during infusion of amino acids, than would have been reabsorbed during control. Also, during AGPS infusion, the dependence of proximal sodium reabsorption on GFR was reduced as evidenced by the fall in the value of the coefficient of determination (r^2; Table 4). In addition, the weak positive relationship between distal sodium reabsorption and GFR was abolished so that distal sodium reabsorption was no longer influenced by GFR.

The rate of distal sodium reabsorption was closely related to the rate of sodium delivery to the distal tubule at all time points in the saline-infused fetuses. The relationships between the amount of sodium delivered to the distal tubule and the amount reabsorbed distally, using data from the eight saline-infused fetuses were:

$$R_{Na,D} = 0.91 \times DD_{Na} - 6.21; r^2 = 0.972, P < 0.001, \quad (6)$$

control**;**

$$R_{Na,D} = 0.90 \times DD_{Na} - 3.45; r^2 = 0.990, P < 0.001, \quad (7)$$

at 2 h;

$$R_{Na,D} = 0.96 \times DD_{Na} - 8.43; r^2 = 0.995, P < 0.001, \quad (8)$$

at 4 h,

where DD_{Na} is the amount of sodium delivered to the distal nephron and $R_{Na,D}$ is the amount reabsorbed distally, both in μmol min^{-1}.

A similar relationship was seen in the control period for the 11 amino acid-infused fetuses, but at 2 and 4 h the regression lines were shifted to the right, indicating that

the percentage of the distal load reabsorbed by the distal nephron decreased during AGPS infusion:

$$R_{Na,D} = 0.80 \times DD_{Na} + 1.24; r^2 = 0.912, P < 0.001, \quad (9)$$

control;

$$R_{Na,D} = 0.81 \times DD_{Na} - 28.49; r^2 = 0.842, P < 0.001, \quad (10)$$

at 2 h;

$$R_{Na,D} = 0.74 \times DD_{Na} - 38.63; r^2 = 0.783, P < 0.001, \quad (11)$$

at 4 h.

Renal acid–base handling

In the saline group, apart from a fall in urinary pH by the end of infusion, there were no changes in urinary acid–base parameters (Table 5). In fetuses given amino acid infusions, although there was a marked increase in bicarbonate reabsorption, fractional bicarbonate reabsorption fell, as was the case for the fractional reabsorption of sodium. Only 2 out of the 11 fetuses had positive net acid excretion during the control period (0.4 and 2.2 μmol min^{-1}). During amino acid infusion there was a large, progressive increase in net acid excretion, largely due to increased excretion of ammonium ions (Table 5). The pH of fetal urine did not change over the

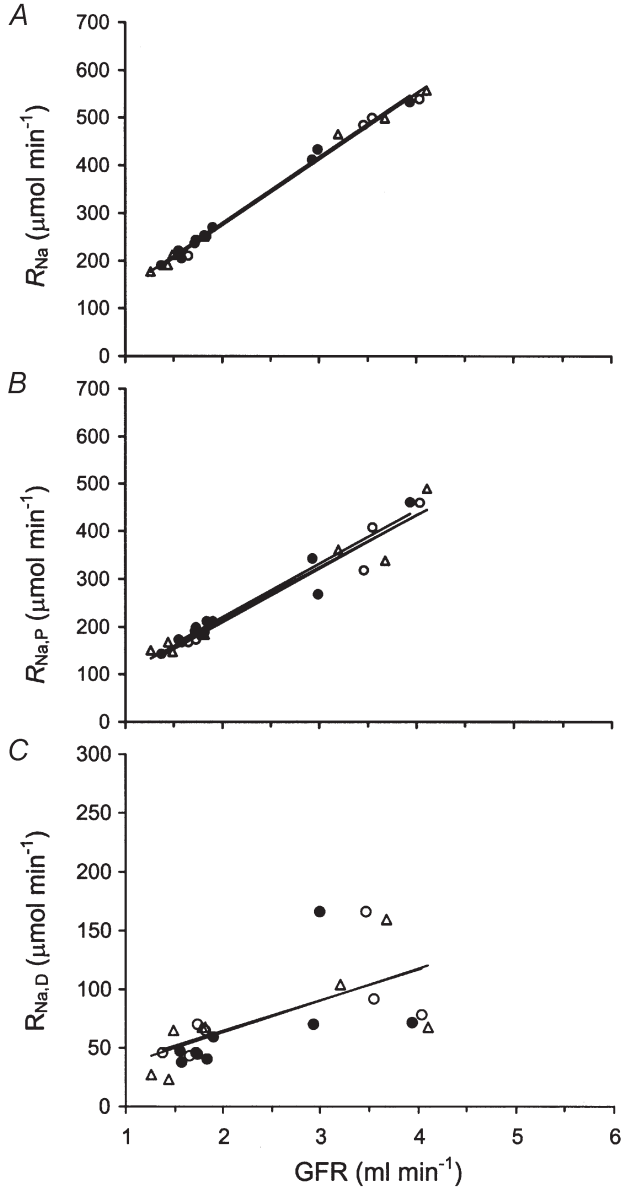

Figure 4. Renal tubular sodium reabsorption before and during saline infusion

Positive relationships in 8 fetuses between GFR (x-axis) and total (R_{Na}; A) proximal ($R_{Na,P}$; B) and distal sodium reabsorption ($R_{Na,D}$; C) (y-axis) during the control period (○) and after 2 h (△) and 4 h (●) of isotonic saline infusion. Linear regression equations describing each relationship and their r^2 values are shown in Table 4.

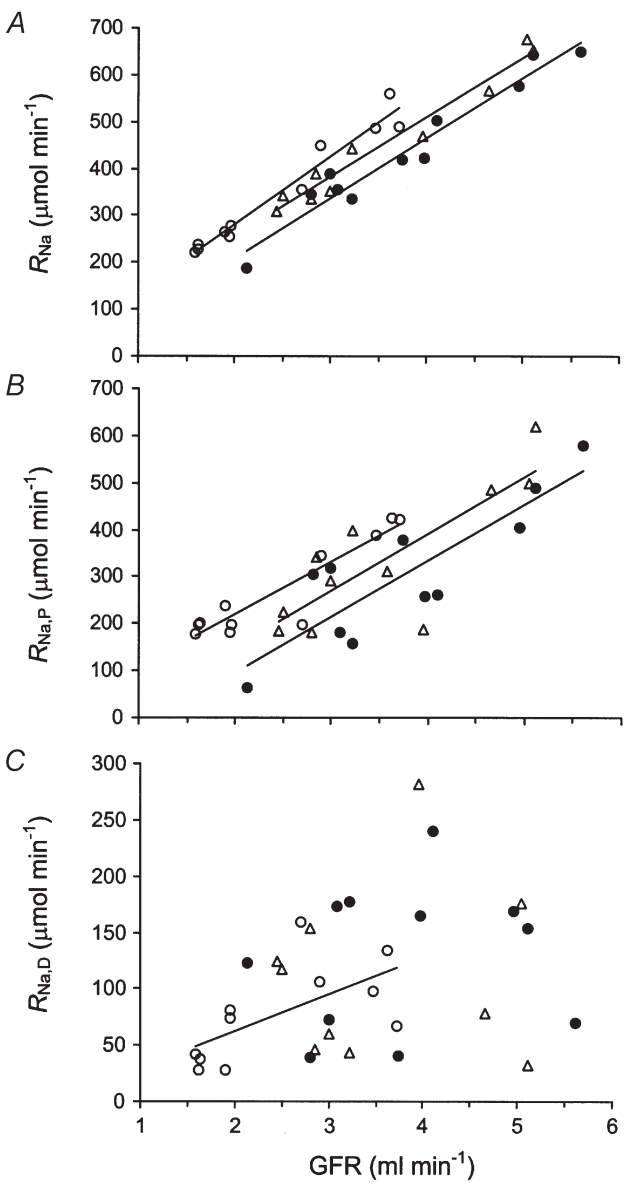

Figure 5. Renal tubular sodium reabsorption before and during a 4 h mixed amino acid infusion

Positive relationships in 11 fetuses between GFR (x-axis) and total (R_{Na}; A) proximal ($R_{Na,P}$; B) and distal sodium reabsorption ($R_{Na,D}$; C) (y-axis) during the control period (○) and after 2 h (△) and 4 h (●) of a mixed amino acid infusion. Linear regression equations describing each relationship and their r^2 values are shown in Table 4.

Table 5. Renal acid–base measurements

	Saline			Amino acids		
	Control	2 h	4 h	Control	2 h	4 h
Arterial pH	7.35 ± 0.01	7.33 ± 0.01	7.32 ± 0.01	7.33 ± 0.01	7.30 ± 0.01 *	7.32 ± 0.02 (10)
Urine pH	6.83 ± 0.19	6.84 ± 0.19	6.68 ± 0.18 ***†††	6.68 ± 0.10	6.71 ± 0.11	7.01 ± 0.14 ***†††
$Filt_{HCO_3}$ (μmol min^{-1})	68 ± 10 (8)	63 ± 11 (8)	62 ± 8 (8)	68 ± 6 (11)	89 ± 7 *** (11)	101 ± 9 ***††† (10)
R_{HCO_3} (μmol min^{-1})	59 ± 10	54 ± 10	54 ± 7	71 ± 9	90 ± 8 **	96 ± 12 ***
FR_{HCO_3} (%)	94.6 ± 2.5	96.2 ± 1.5	96.1 ± 1.7	97.0 ± 0.5	94.9 ± 0.9	90.4 ± 2.0 ***††
E_{HCO_3} (μmol min^{-1})	2.7 ± 1.1	1.9 ± 0.5	1.8 ± 0.7	2.3 ± 0.6	5.0 ± 1.3 ***	9.2 ± 1.3 ***†††
E_{TA} (μmol min^{-1})	0.6 ± 0.3	0.5 ± 0.3	0.5 ± 0.3	0.3 ± 0.1	0.8 ± 0.2	0.7 ± 0.4
E_{NH_4} (μmol min^{-1})	1.4 ± 0.5 (6)	1.3 ± 0.4	1.3 ± 0.3	1.2 ± 0.2	14.4 ± 0.6 ***	32.2 ± 1.7 ***††† (7)
NEA (μmol min^{-1})	0.3 ± 1.2 (6)	−0.1 ± 0.9	0.0 ± 1.1	−0.7 ± 0.7	10.2 ± 1.5 ***	23.1 ± 3.1 ***†††

Means ± S.E.M. during the control period and after 2 and 4 h of infusion of either isotonic saline ($n = 7$, unless stated in parentheses) or amino acids ($n = 7$ during control and at 2 h, $n = 6$ at 4 h, unless stated in parentheses). $Filt_{HCO_3}$, R_{HCO_3} and FR_{HCO_3}, filtered load and absolute and fractional reabsorption of bicarbonate, respectively; E_{HCO_3}, E_{TA} and E_{NH_4}, excretion of bicarbonate, titratable acid and ammonium, respectively; NEA, net excretion of acid. $^{**}P < 0.01$, $^{***}P < 0.005$ *vs.* control; ††$P < 0.01$, †††$P < 0.005$ *vs.* 2 h (Student-Newman-Keuls test).

first 2 h of AGPS infusion, but became less acidic during the last 2 h.

Maternal variables

There were no changes during either saline or amino acid infusion in any of the maternal variables measured (mean arterial pressure, heart rate, P_{O_2}, P_{CO_2}, pH, haematocrit, plasma osmolality and plasma concentrations of sodium, potassium and bicarbonate). Control values in the saline group were 98 ± 5 mmHg, 122 ± 10 beats min^{-1}, 108.4 ± 3.6 mmHg, 35.8 ± 0.7 mmHg, 7.47 ± 0.01, 25.0 ± 1.5 %, 293 ± 4 mosmol kg^{-1}, and 147 ± 2, 3.7 ± 0.1 and 25.2 ± 0.5 mmol l^{-1}, respectively. In the amino acid group, control values were 87 ± 2 mmHg, 104 ± 6 beats min^{-1}, 105.5 ± 3.3 mmHg, 38.3 ± 1.7 mmHg, 7.45 ± 0.01, 24.6 ± 2.0 %, 297 ± 4 mosmol kg^{-1}, and 159 ± 4, 4.3 ± 0.2 and 25.5 ± 0.8 mmol l^{-1}, respectively.

DISCUSSION

In this study the fetal kidney at 0.8 of gestation exhibited a marked, sustained response to elevated plasma levels of amino acids. This response was characterized by large increases in GFR and filtration fraction, an osmotic diuresis, a natriuresis, kaliuresis, greatly increased ammonium excretion and increases in both proximal and distal tubular sodium reabsorption. Furthermore, plasma renin levels were suppressed.

Renal haemodynamics

In the present study, increases of up to 20-fold in fetal plasma levels of the individual amino acids infused were associated with increases in GFR of up to 55 %. Others have defined several indices of renal function following acute protein loading in adults, based on the GFR response: the filtration capacity (the maximum GFR reached), the renal reserve (the absolute increase in GFR; Bosch *et al.* 1984) and the percentage increase in GFR from control levels (Woods, 1993*a*). In the present study the 'renal reserve' was the same in all fetuses. That is, each fetus responded to the given amino acid stimulus by a similar absolute increase in GFR ($GFR_{inf} = 1.03GFR_{control} + 1.24$; eqn (4)). It is therefore not surprising that the increase in GFR during infusion expressed as a percentage of control was inversely related to control GFR ($\Delta GFR_{\%control} = e^{((0.89/GFR_{control}) + 4.65)}$; eqn (5)). It also explains why the 'filtration capacity' of each fetus depended on its control GFR (eqn (4)). Given the large increases in plasma amino acid levels during infusion it was surprising that the increment in GFR was not a more varied response based on individual differences in the structural and functional maturity of the kidney. Indeed, because we gave the same dose of AGPS to each fetus, one might expect the increase in GFR to be larger in smaller fetuses, because they would have received a larger dose per kilogram body weight. We did not attempt to construct a dose–response curve and so

cannot speculate on whether the increment in GFR in these fetuses was close to maximal, or whether a fetus with a high GFR during control was operating near its maximum. Certainly though, a low GFR during control did not appear to limit the GFR response to the amino acid dose given.

Although in the present study the fetal kidney responded to amino acids in a similar manner to the adult kidney in that GFR increased, the RBF response was very different. This is the first time that the effects of amino acid infusion on blood flow to the fetal kidney have been studied. Although there was an increase in RBF it was small and was not sustained, unlike the rise in GFR which persisted throughout the 4 h infusion. Thus the filtration fraction rose (Fig. 2). Most studies on the effects of a protein meal or an amino acid infusion on adult renal haemodynamics report that RBF increases in parallel with, and in proportion to, the increase in GFR, so that the filtration fraction does not change (Hostetter, 1986; Woods *et al.* 1986; Castellino *et al.* 1987; Premen, 1989). In adult animals these increases in RBF occur in the absence of a change in blood pressure or renal perfusion pressure (Premen, 1989). Instead, renal vascular resistance is usually reduced, and this, combined with the lack of change in filtration fraction, suggests that amino acids stimulate increases in GFR and RBF in the adult via renal vasodilatory mechanisms, presumably vasodilatation of the afferent arteriole (Hostetter, 1986; Thomas *et al.* 1994).

In the present study, in contrast to the renal vasodilatation which occurs during amino acid infusion in the adult animal, it is likely that there was vasoconstriction of the efferent arteriole. This is consistent with the large increases in GFR and filtration fraction that were observed in these amino acid-infused fetuses. The small increase in RBF at 2 h, and the fact that RBF did not fall, suggests that a small degree of afferent arteriolar vasodilatation also occurred. Thus there are striking differences between the adult and fetal renal haemodynamic responses to amino acid infusion, both in terms of the site and the nature of the vascular response. Resting renal vascular resistance is high in the fetal sheep kidney (Lumbers, 2000), which suggests that the vasoactive milieu of the fetal kidney differs from that of the adult and perhaps explains their very different vascular responses to amino acids.

Although the increase in GFR in the present study could not have been caused by increases in renal blood flow, it may have been due to other effects of the infusion. Fetuses probably became volume expanded during amino acid infusion, as evidenced by the fall in haematocrit, plasma renin levels and in plasma sodium and chloride levels. In addition to isotonic saline, 0.32 mmol min^{-1} of osmotically active amino acids were infused into the vascular space. Taking into account the rise in osmolar excretion, about 50 mosmol were added to the extracellular space. By the end of infusion this should have raised the plasma osmolality by about 30 mosmol kg^{-1} (Gibson & Lumbers, 1995); however, plasma osmolality did not change. Thus water must have been transferred across the placenta to the fetus. This volume expansion may have contributed to the increased GFR during amino acid infusions by diluting plasma proteins and hence reducing glomerular capillary oncotic pressure.

Volume expansion would also explain why plasma renin levels were suppressed. This fall in plasma renin means that, although angiotensin II plays an important role in maintaining fetal GFR, partly via its actions on the efferent arteriole (Lumbers *et al.* 1993), it is unlikely that it was responsible for the rise in GFR during amino acid infusion.

In adult humans and dogs, plasma glucagon levels rise following a meat meal (Ando *et al.* 1989), and, in adult humans, infusions of glucagon to raise plasma levels to those expected after a meat meal stimulated similar increases in GFR and RBF to those caused by a protein or amino acid load (Hirschberg *et al.* 1988). However, even though plasma glucagon levels increased during I.V. infusion of glycine to late gestation fetal sheep (Bassett *et al.* 1983), glucagon does not appear to increase GFR in the fetus. Pharmacological doses of glucagon to late gestation fetal sheep had no effect on many aspects of renal function including GFR, urine flow rate and sodium excretion (Moore & Lumbers, 1992).

The almost 4-fold increase in cortisol levels during amino acid infusion may have contributed to the rise in GFR during AGPS infusion, as GFR has been shown to increase in fetal sheep following I.V. injections of cortisol (Hill *et al.* 1988). In addition, increases in plasma cortisol are often paralleled by increases in arginine vasopressin (AVP) (Wintour *et al.* 1985). The increase in fetal GFR in the amino acid group may therefore have depended on an increase in plasma AVP (Gibson & Lumbers, 1993); however, AVP levels were not measured.

Tubular reabsorption

The large rise in GFR and hence in filtered load (Table 3) might be expected to be accompanied by increases in sodium reabsorption in an effort to maintain glomerulotubular balance. In the fetus, maintenance of glomerulotubular balance depends on increased sodium reabsorption by both the proximal and distal tubule (Lumbers *et al.* 1988). Although both proximal and distal sodium reabsorption markedly increased during amino acid infusion, in neither part of the nephron was this sufficient to maintain glomerulotubular balance. Thus fractional proximal tubular reabsorption decreased substantially, so that the distal delivery of sodium increased, and the fraction of this distally delivered load that was reabsorbed also fell (Table 3). The linear relationship between distal sodium reabsorption and GFR, seen in control and in

saline-infused fetuses (Figs 4 and 5), and by us in earlier studies (Lumbers *et al.* 1988) was lost. Furthermore, this inability of the renal tubules to handle the increased filtered load during amino acid infusion led to large increases in electrolyte excretion, and this, perhaps in combination with volume expansion, resulted in fetuses becoming hyponatraemic and hypochloraemic.

It is well known that in fetal sheep the proximal tubule is immature, and it is not unusual for proximal glomerulotubular balance to be disrupted in the fetus during acute increases in GFR, for example during infusion of AVP (Gibson & Lumbers, 1993), cortisol (Hill *et al.* 1988) and atrial natriuretic peptide (Shine *et al.* 1987). The increased variability of the relationship between GFR and proximal or distal sodium reabsorption during amino acid infusion in the present study (Table 4) indicated that some fetuses were more able than others to increase sodium reabsorption when GFR increased. This might reflect individual variation in the maturity of tubular reabsorptive processes. Immaturity of amino acid transporters in the proximal tubule at the stage of gestation we studied might have limited the extent to which amino acid transport, and hence sodium cotransport, could increase. Indeed, fractional amino acid reabsorption is lower in human infants than in children or adults (Brodehl & Gellissen, 1968).

The lack of a marked increase in renal blood flow in the present study may have imposed another restriction on proximal tubular function by limiting oxygen supply, as only ~2–3 % of the combined cardiac output is distributed to the fetal kidney (Rudolph & Heymann, 1970). Amino acid reabsorption involves active transport and therefore increases proximal tubular oxygen requirements, which in the adult are met by increased renal blood flow (Woods *et al.* 1986). Incomplete reabsorption of amino acids by the proximal tubule may also have exerted an osmotic diuretic effect. In addition, volume expansion may have hindered proximal reabsorption by reducing peritubular capillary oncotic pressure, which would account for the rightward shift in the reabsorption-GFR relationships shown in Figs 5*A* and 5*B*.

Acid–base homeostasis

In fetuses infused with amino acids there was a compensated metabolic acidosis, as evidenced by the transient, small decrease in arterial pH, doubling of plasma lactate levels, fall in bicarbonate levels and the large increases in ammonium excretion and bicarbonate reabsorption. Not all of these changes were related to acid–base homeostasis, however, as the increase in GFR meant that filtered bicarbonate was increased but, as occurred with sodium, fractional bicarbonate reabsorption fell. Despite this, renal losses only partly account for the fall in plasma bicarbonate levels. At 2 h, fetuses were excreting an extra 2.3 μmol min^{-1} of bicarbonate above control levels, but an extra 13.6 μmol min^{-1} of new bicarbonate was being added to the blood as a result of increased ammonium and titratable acid excretion. Taking these excretion rates at 2 h as average rates for the entire infusion, by 4 h an extra 2.7 mmol bicarbonate had been added to the fetal circulation by the kidneys. In a 2.5 kg fetus with an extracellular volume of 630 ml kg^{-1}, this should raise plasma bicarbonate levels by 1.7 mmol l^{-1}. Instead, mean plasma bicarbonate concentrations fell by 1.4 mmol l^{-1}. Therefore large amounts of bicarbonate were consumed in buffering.

In previous studies from this laboratory, infusion of 17–48 mmol of HCl into fetal sheep caused about a 3-fold increase in titratable acid, nearly a 3-fold increase in urinary ammonium excretion and an increase in net acid excretion, which was negative prior to infusion of HCl, to between 7 and 8 μmol min^{-1} (Kesby & Lumbers, 1988). In the present study, approximately 60 mmol of amino acids had been infused by 4 h. Both ammonium and net acid excretion increased, so that by 4 h they were about 30 times greater than control values and 23 μmol min^{-1}, respectively. These findings demonstrate that the capacity of the fetal sheep kidney at this stage of gestation to increase renal ammonium and net acid excretion is considerably greater than that shown in our previous studies.

To conclude, the fetal kidney responded to infusions of amino acids by considerable, sustained increases in GFR, but no sustained increase in RBF, indicating that efferent arteriolar vasoconstriction occurred. This was a very different response from the renal vasodilatation that occurs in adult animals during amino acid infusion. Fractional sodium reabsorption fell markedly despite substantial increases in both proximal and distal sodium reabsorption; the failure to maintain proximal glomerulotubular balance was partly due to the osmotic diuresis that occurred, and possibly also to a reduction in capillary oncotic pressure secondary to volume expansion. Plasma renin levels were suppressed, possibly due to volume expansion, and fetuses developed a compensated metabolic acidosis. The magnitude of the sustained response of the fetal kidney to amino acids indicates that a remarkable functional capacity is present before birth.

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